PCT WELTORGANISATION FÜR GEISTIGES EIGENTUM
Internationales Büro

INTERNATIONALE ANMELDUNG VERÖFFENTLICHT NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT)

(51) Internationale Patentklassifikation 6 :		(11) Internationale Veröffentlichungsnummer: WO 95/17167
A61K 9/16, 39/39	A1	(43) Internationales Veröffentlichungsdatum: 29. Juni 1995 (29.06.95)

(21) Internationales Aktenzeichen: PCT/CH94/00242

(22) Internationales Anmeldedatum: 23. December 1994 (23.12.94)

(30) Prioritätsdaten:
3849/93-6 23. December 1993 (23.12.93) CH

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(81) Bestimmungsstaaten: AT, AU, BB, BG, BR, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, europäisches Patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Veröffentlicht

Mit internationalem Recherchenbericht.

Vor Ablauf der für Änderungen der Ansprüche zugelassenen Frist. Veröffentlichung wird wiederholt falls Änderungen eintreffen.

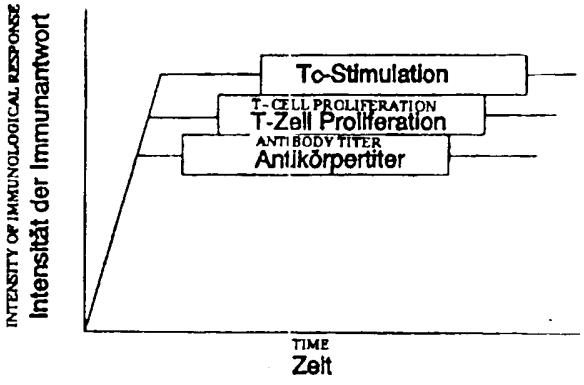
2156718

(54) Title: IMMUNOLOGICAL RESPONSE POTENTIATION PROCESS

(54) Bezeichnung: VERFAHREN ZUR POTENZIERUNG DER IMMUNOLOGISCHEN ANTWORT

(57) Abstract

An immunological response potentiation process is disclosed for synthetic or genetically engineered antigens having low immunogenicity. The antigen is embedded into biodegradable microparticles, these antigen-loaded microparticles are dispersed in a biodegradable medium which triggers when it is parenterally administered a potentiated antibody, T_H-lymphocyte and T_c-lymphocyte response, as compared to an aqueous antigen solution. The extent of immunological potentiation is at least comparable with that attained by IFA compositions. Linear B-T_H-cell epitopes, linear T_c-cell epitopes, dimers and multimers of said epitopes, as well as their mixtures, are used as low immunogenicity antigens. The microparticles are based on biodegradable biopolymers such as polyester, polyanhydride, polyorthoester. By mixing microparticles with different wettabilities, swellabilities, release and biodegradation times, the most intense and longest immunological potentiation is achieved. This process is useful for immunising human beings and animals against diseases caused by viruses, bacteria, protozoa or tumour cells.



Claims:

1. A process for the potentiation of the immunological response of humans and animals to synthetic antigens, characterized in that the synthetic antigen is first embedded into biodegradable spherical microparticles, that subsequently the microparticles loaded with the synthetic antigen are suspended in a dispersion medium and that this preparation is parenterally administered, by means of which a potentiated immune response is triggered.

2. A process in accordance with claim 1, characterized in that a compound consisting of a simple chain with at least one B-cell epitope and at least one T_H-cell epitope is used as the synthetic antigen.

3. A process in accordance with claim 1, characterized in that a compound consisting of a repetitively covalently linked B-cell epitope and a T_H-cell epitope is used as the synthetic antigen.

4. A process in accordance with claim 1, characterized in that a compound consisting of a cytotoxic T-cell epitope (T_C) is used as the synthetic antigen.

5. A process in accordance with claim 1, characterized in that a mixture of at least one T_H- and at least one T_C-epitope is used as the synthetic antigen.

6. A process in accordance with one of claims 1 to 5, characterized in that the synthetic antigen contains B- and T-cell epitopes derived from protozoa or viruses or bacteria or tumor cells.

7. A process in accordance with one of claims 1 to 5, characterized in that the synthetic antigen contains B- and T-cell epitopes derived from protozoa or viruses or bacteria or tumor cells or an arbitrary combination thereof.

8. A process in accordance with one of claims 1 to 7, characterized in that the spherical microparticles are constructed from a biocompatible, biodegradable biopolymer, wherein the biopolymer is derived from the group of poly (lactic acid), poly (glycolic acid), poly (lactic-coclykolic acid), polycaprolactone, poly (hydroxybutyric acid), poly (hydroxybutyric-covaleric acid), polyorthoester or from the polyanhydrides.

9. A process in accordance with claim 8, characterized in that the biopolymers are derived from at least two groups.

10. A process in accordance with one of claims 1 to 9, characterized in that an aqueous or oily lecithin solution or an aqueous-oily lecithin emulsion is used as the dispersion medium.

11. A process in accordance with one of claims 1 to 9, characterized in that a microemulsion is used as the dispersion medium.

12. Use of the process in accordance with one of claims 1 to 11 for immunizing humans and animals against diseases caused by viruses, bacteria, protozoa or tumor cells.

PCT/CH94/00242 WO 95/17167

Specification:

Immunological Response Potentiation Process

The instant invention relates to a process for potentiating the immunogenicity of synthetic, weakly immunogen antigens. In what follows, synthetic, weakly immunogenic antigens are understood to be compounds having peptide or protein structures which are produced either chemically or by means of recombinant DNA technology and which, following parenteral administration in an aqueous solution or in the form of an aluminum adsorbate, only trigger an unimportant immunological response with very low antibody titers and lacking or only small T-cell proliferation. This group of antigens will hereinafter be called synthetic antigens for the sake of simplicity. By definition the immune response to the synthetic antigens herein described is therefore negligible if they are administered in an aqueous solution. Incomplete Freund's adjuvant (IFA) is used as an experimental reference preparation. IFA is a water in oil (W/O) preparation which is known to stimulate the humoral as well as the cellular immune response. However, IFA can only be used for testing purposes because of strong, undesirable side effects. By the term vaccine, formulations will hereinafter be understood which contain, in addition to the antigen, substances which themselves perform the function of purely inactive ingredients or an immunopotentiating function or even a combination of both functions. Purely inactive ingredients are, for example, water for dissolving the antigen for the parenteral administration,

anti-microbial, isotonic and pH-stabilizing inactive ingredients. Often immunipotentiating substances are called adjuvants, comprising, for example, insoluble aluminum salts (-phosphates, -hydroxides), certain lipopolysaccharides, muramyl peptide, trehalose compounds, several cytokines, such as interleukine 1, lipophilic block copolymers (poloxamers). However, the experimental reference preparation incomplete Freund's adjuvant and several administration forms for vaccines, such as liposomes, emulsions, nano-capsules, also have adjuvant properties. These forms of administration not only cause the formation of an antigen deposit in vivo, but also have immune-stimulating properties.

The development of new vaccines and the improvement of existing vaccine formulations has gained in importance and urgency in the past years (E. Eppstein et al., New Adjuvants for Vaccines Containing Purified Protein Antigens, Advances in Drug Delivery Review 4, 233 - 253, (1990)). The production of synthetic antigens as well as the development of suitable adjuvant formulations and forms of administration which increase the immunogenicity of weakly immunogenic compounds are in the forefront. The target of the development of new antigens is, on the one hand, diseases, such as AIDS, malaria, tuberculosis, cholera, hepatitis A, cancerous diseases, against which there are as yet no or only insufficiently active vaccines; on the other hand efforts are directed toward the replacement of the antigens contained in the traditional vaccines, such as inactivated viruses, bacteria or toxoids, by low-molecular peptides and proteins which are easier to produce and to clean and can be better characterized and which have the antigen regions of the actual infection agents in their structure. Such antigen peptides

and proteins can be obtained at high purity biochemically or by means of recombinant DNA technology. This new generation of synthetic antigens has peptide sequences (epitopes) in its chemical structure which stimulate antigen-specific T_H- (helper), T_C- (cytotoxic) and B-lymphocytes. Here the so-called T_H-, T_C- and B-cell epitopes can each be individually present or can be covalently linked into a chimeral B-T epitope. Since these gene-technologically or chemically produced antigens have in general lower molecular weights of approximately 500 to 2,000, their immunogenicity is very weak in contrast to toxoids of molecular weights of 50,000 to 150,000 or in contrast to particular antigens, such as inactivated viruses and other microorganisms.

Strategies for immunogenicity potentiation of synthetic antigens known up to now are based on increasing in a first step the molecular weight of these antigens by covalent linking and, in a second step, to insert these higher molecular structures into immunopotentiating formulations.

It is known that an increase of the molecular weight can be achieved in that the synthetic antigen is covalently bonded to high-molecular carrier proteins, such as diphtheria and tetanus toxoids, bovine serum albumin, rock limpet hemocyanin. In connection with the antigen carrier constructs, the employment of very expensive and relatively impure foreign organisms, the necessity for reactive and relatively toxic agents for the covalent linking of the antigen and the carrier protein and the difficulty of purification as well as identity and purity tests of these compounds are disadvantageous. On the other hand, it has also been proposed to increase the molecular weight of B-T epitopes by covalently linking them in a sort of branch structure

to form multimers (J. P. Tam, Y.-A. Lu, Proceedings of the National Academy of Sciences of the USA 86, 9084 - 9088 (1989)). These constructs were called multiple antigen peptides, MAP in short.

It is further known that the combination of a B- and T_H-epitope is essential for an antibody formation to take place, and that the combination of a T_C-epitope with a T_H-epitope can improve the cytotoxic lymphocyte response, also called CTL response, after administration in IFA (C. Widman et al., J. Immunol. Methods 155, 95 - 99 (1992)).

Different immunopotentiating formulations for such weak immunogen antigens and their higher-molecular constructs are described in PS-EP-A1-513,861. O/W emulsions containing immune stimulants are a primary part thereof. Their inherent thermodynamic instability, which can be reflected as the appearance of coalescence during storage, is disadvantageous in connection with these roughly-dispersed or colloid-dispersed systems. In addition, the components of such liquid-dispersed formulations suffer from chemical changes, such as oxidation and hydrolysis. The described formulations furthermore mostly require immune stimulants, such as muramyl peptides, which are not quite harmless toxicologically. Finally, these formulations do not show any long time effects at all. To obtain protection by vaccination over several years it is therefore necessary to inject these vaccine formulations three to four times in accordance with a defined vaccination plan (so-called "booster" injections).

Furthermore, a system for the immune potentiation of antigens is known from the international publication WO 92/19263-A1, which employs so-called biodegradable microspheres, also

called microcapsules or microparticles. It is disadvantageous with this method that the immune potentiation is mainly observed in the gastro-intestinal mucuous membranes and therefore will probably show an effect only on relative few pathogens (so-called enterophatogenic microorganisms). The fact that the microparticles are administered in the duodenum and cannot be administered or taken perorally precludes a practical use, at least in humans. It furthermore appears in accordance with PS-EP-A2-333,523 that a balanced amount of fine (1 to 10 μm) and coarser grained (20 to 50 μm) of microparticles seems to be an important prerequisite for the immune-potentiating effect. Thess requirements for the grain size range of the microparticles represent an additional effort during production and processing of the microparticles, which appears to be disadvantageous.

It is the object of the invention to embed a synthetic antigen by means of specific biopolymers into biodegradable microparticles, to suspend these microparticles in a dispersion medium and to administer them parenterally, by means of which a potentiation of the systemic immunological response is caused.

In accordance with the invention this object is attained by means of a process in accordance with the wording of claims 1 to 12. Exemplary embodiments for this are described in Examples 1 to 6. The process of the invention will be explained in detail below by means of Figs. 1 to 7. Shown are in:

Fig. 1, a schematic representation of the immune potentiation in accordance with the invention,

Fig. 2, antibody titers after a single administration of quick-release microcapsules containing MAP and an IFA formulation,

Fig. 3, antibody titers after a single administration of slow-release microcapsules containing MAP and an IFA formulation,

Fig. 4, antibody titers after a single administration of a mixture of quick- and slow-release microcapsules containing MAP and an IFA formulation,

Fig. 5, antibody titers after a triple administration of quick-release microcapsules containing MAP and an IFA formulation,

Fig. 6, Proliferative T-lymphocyte response after a single administration of different, MAP-containing microcapsules and an IFA formulation,

Fig. 7, T_C-response after a single administration of a mixture of quick-release microcapsules containing respectively a synthetic T-$_C$-antigen and a corresponding MAP.

1. Embedding Synthetic Antigen in Biodegradable Micro-Particles.

The starting point of the process are synthetic antigens in accordance with the definition in the preamble of this patent, which contain in their known chemical structure at least one defined epitope of a pathogenic microorganism which can be recognized by the immune system. In this case the epitope can be a B-cell epitope, a T_H-cell epitope, a T_C-epitope or an arbitrary mixture of these epitopes. The so-called multiple antigen peptides (MAP), alone or in combination with a T_C-epitope, preferably constitute the starting point of the process. The origin of the epitopes includes bacteria, viruses, protozoa and tumor cells. In accordance with the invention, the synthetic antigen is embedded into biodegradable microparticles. It is

essential for the invention that biopolymers with specific physico-chemical properties are selected for producing the biodegradable microparticles. Important properties are wettability, insolubility, expandability and biodegradability of the biopolymers and the spherical microparticles produced therefrom in aqueous media and physiological liquids. The extent of the expandability of the biopolymers and their biodegrading time importantly determine the release kinetics of the antigens from the microcapsules. It has now been surprisingly found that these release kinetics also affect the progression over time of the immune response. Examples of such biopolymers of varying wettability, expandability and biodegrading time are poly (lactic acid), poly (lactic-co-clykolic acid), pcly (hydroxybutyric acid), poly (hydroxybutyric-covaleric acid), poly (caprolactone). Embedding of the synthetic antigen into the biopolymer is performed by means of various known methcds such as spray-drying, solvent evaporation or co-azervation. Antigen-loaded, spherical microparticles of a size of 1 to 200 μm result from this.

2. Suspension in an Dispersion Medium

In the second step, the antigen-loaded microparticles in accordance with the invention are placed into a dispersion medium which is suitable for the parenteral administration of the microparticles. In this connection it is essential for the invention that the dispersion medium be biocompatible and biodegradable and in addition have advantageous properties for potentiating the immune response. Such advantageous dispersion media are, for example, aqueous or oily solutions of lecithin or

aqueous-oily emulsions with lecithin at a concentration range of 0.1 to 20%, preferably 2 to 10%. Further suitable dispersion media are so-called microemulsions comprising a water, oil, tenside and co-tenside component. Biocompatible and biodegradable substances, such as natural or synthetic mono-, di- and triglycerides, lecithin, poloxamers and polysorbates are used for this. The dispersion media mentioned are distinguished by surprisingly good wetting and suspension properties of the biodegradable microparticles. These wetting and suspension properties are for example considerably better than those of the usually employed dispersion media, such as carboxymethyl cellulose or polysorbate. The dispersion of the microparticles in the dispersion medium can be performed simply by shaking, by means of which an injectable preparation is created.

3. Administration

The antigen-loaded microparticles suspended in the suspension medium are administered parenterally, wherein this administration can take place once or several times at defined intervals. The latter form of administration is known under the term "booster". The first and second booster dose can be administered 1 to 4 weeks and 3 to 6 months after the initial injection, for example. A potentiated immune response lasting for several months is triggered following the single or multiple administration of the formulations in accordance with the invention.

4. Achieving the Potentiated Immune Response

The potentiation of the immune response is generally measured in BALB/c mice after a single, in exceptional cases also after a triple parenteral administration of the microparticles loaded with synthetic antigens in accordance with the invention. MAP, produced from a universal T_H-epitope of the tetanus toxin (sequence 947 to 967) and a B-cell epitope of the repetitive region of the circumsporozoite protein of Plasmodium berghei, and a T_C-epitope of the circumsporozoite protein of Plasmodium berghei (sequence 252 to 260) is used as synthetic model antigen (S. Demotz et al., J. of Immunology 142, 394 to 402 (1989); P. Romero et al., Nature 341, 323 (1989); J.L. Weber et al.; Exp. Parasitology 63, 295 (1987)). The intensity and length of the immune potentiation is measured by means of the specific antibody titers, the T-lymphocyte proliferation and the specific cytotoxic T-lymphocyte activity. These three parameters are determined in accordance with known immunological methods.

Fig. 1 illustrates schematically the relevant parameters of the immune potentiation achieved by means of the process in accordance with the invention. In the instant patent an immune potentiation means that the intensity during the progression over time of the immunological response as a result of an administered synthetic antigen has been potentiated in respect to an aqueous antigen solution and has been potentiated in a comparable or increased amount in respect to an IFA formulation on the levels of the antibody titer, the T-cell proliferation and the T_C-stimulation.

The possibility arises from this by means of mixing biopolymers of different wettability, expandability and biodegrading time to potentiate the humoral antibody response as well as the cellular T-lymphocyte response to an extent which is comparable or even greater then the potentiation achieved by means of incomplete Freund's adjuvant. In addition, the immune responses in accordance with this process can be time-controlled and are extended over several weeks in contrast to IFA and aqueous solutions.

Because of the custom-made properties of the biodegradable microparticles used, the process here described makes possible a directed potentiation of the humoral and cellular immune responses to synthetic antigens, in particular the so-called MAP, which can be controlled in its progression over time. Moreover, the process has the extraordinary advantage that it is possible to stimulate cytotoxic T-lymphocytes in addition to the directed and potentiated stimulation of B- and T_H-lymphocytes, because of which it is also possible to successfully provide immunization against viruses, protozoa and tumor cells in particular. It was surprisingly possible to demonstrate this cytotoxic stimulation of T-lymphocytes here for the first time. In contrast to the immune potentiation described in PS EP-A2-333,523 and PCT WO 92/19263, the instant one is primarily systemic, i.e. not mucosal, and can be controlled in intensity as well as duration or progression over time. Furthermore, a narrow, exactly defined particle size distribution is not required for the immune potentiation, which entails technological advantages.

The process here described is used for the immunization of humans and animals against diseases caused by bacteria, viruses,

protozoa and tumor cells. The immunization against viruses, protozoa and tumor cells in particular which, with the use of conventional vaccines, can only be achieved in an unsatisfactory way, i.e. insufficiently and by accepting undesired side effects, represents a main application of this method. The stimulation of the cytotoxic T-cells by means of the process in accordance with the invention as well as the immune response lasting over an extended period of time constitute the basis for this application.

Example 1 describes the potentiation of the antibody response to the branched multiple antigen peptide identified as P30B2, which is constructed from a universal T_H-cell epitope of the tetanus toxin (sequence 947 to 967) and a B-cell epitope of Plasmodium berghei: 0.02 g P30B2 were dissolved in 2.00 g water and this solution was subsequently dispersed with the aid of an ultrasonic generator in a solution of 2.0 g poly(d, 1-lactic acid co-glycolic acid) 50:50 (Resomer 502, Boehringer Ingelheim) in 40.0 g dichloromethane. Spherical microparticles (RG502) were produced from this dispersion by means of spray drying. Microparticles loaded with antigen were subsequently suspended in a 5% sterile solution of egg lecithin (Ovothin 170, Lukas Meyer, D-Hamburg) by shaking. This suspension was subcutaneously injected into a group of 8 BALB/c mice in respective amounts of 0.5 ml. The amount of antigen injected into each mouse was 30 µg. A second group of 8 BALB/c mice were immunized with the same amount of antigen in incomplete Freund's adjuvant (IFA) as a control. The antibody titers were determined by means of ELISA.

Fig. 2 shows the progression over time of the immune potentiation by RG502 in comparison to IFA associated with Example 1. The antibody titers obtained from RG502 and IFA are comparable

to each other during the first 15 weeks following immunization. After that the titers induced by IFA drop off, while the titers induced by the microcapsules remain constant over at least 28 weeks. Antibody titers of 1 to 2 · 10^3 were already obtained two weeks after administration with a hydrophilic, strongly expandable, quick-release and quickly bicdegradable biopolymer such as PLGA 50:50, and remain constant cver a period of at least 28 weeks. In contrast thereto, the titers measured following a one-time administration of an IFA preparation drop off already after 15 weeks and after 28 weeks are only at 2 · 10^2.

Example 2 describes the potentiation of the antibody response to the branched multiple antigen peptide of the designation P30B2 (in accordance with Fig. 1), constructed from a universal T_H-cell epitope of the tetanus toxin (sequence 947 to 967) and a B-cell epitope of the repetitive region of the circumsporozoite protein of Plasmidium berghei: 0.02 g P30B2 were dissolved in 2.00 g water and this solution was subsequently dispersed with the aid of an ultrasonic generator in a solution of 2.0 g poly (d, 1-lactic acid) (Resomer 206, Boehringer Ingelheim) in 40.0 g dichloromethane. Spherical microparticles (R206) were produced from this dispersion by means of co-azervation, induced by the addition of silicon oil. Microparticles loaded with antigen were subsequently suspended in a 5% sterile solution of egg lecithin (Ovothin 170, Lukas Meyer, D-Hamburg) by shaking. This suspension was subcutaneously injected into a group of 8 BALB/c mice in respective amounts of 0.5 ml. The amount of antigen injected into each mouse was 30 µg. A second group of 8 BALB/c mice were immunized with the same amount of antigen in

incomplete Freund's adjuvant (IFA) as a control. The antibody titers were determined by means of ELISA.

Fig. 3 shows the progression over time of the immune potentiation by R206 in comparison to IFA associated with Example 2. The antibody titers obtained from the hydrophobic, weakly expandable, slow-release and slowly biodegradable R206 continuously rise during the first 12 weeks and then reach the level which had been achieved with IFA already 2 weeks after immunization. While the IFA titers drop again steadily after approximately 15 weeks, the R206 titers remain constant over a period of at least 28 weeks.

Example 3 describes the potentiation of the antibody response to the branched multiple antigen peptide of the designation P30B2 (in accordance with Fig. 1), constructed from a universal T_H-cell epitope of the tetanus toxin (sequence 947 to 967) and a B-cell epitope of the repetitive region of the circumsporozoite protein of Plasmidium berghei: analogously to Example 1, P30B2 was inserted into poly (d,1-lactic acid co-glycolic acid) 75:25 (Resomer RG752, Boehringer Ingelheim) and processed into spherical microparticles (RG752). Identical amounts of microparticles containing RG752, RG502 (from example 1) and R206 (from Example 2) were suspended in a 5% sterile solution of egg lecithin (Ovothin 170, Lukas Meyer, D-Hamburg) by shaking. This suspension was subcutaneously injected into a group of 8 BALB/c mice in respective amounts of 0.5 ml. The amount of antigen injected into each mouse was 30 µg. A second group of 8 BALB/c mice were immunized with the same amount of antigen in incomplete Freund's adjuvant (IFA) as a control. The antibody titers were determined by means of ELISA.

Fig. 4 shows the progression over time of the immune potentiation by a mixture of RG506, RG752 and R206 in comparison to IFA associated with Example 3. The antibody titers obtained from this microcapsule mixture of fast- and slow-release biopolymers rises quickly and reaches a level higher by a factor of 2.5 than the antibody titers achieved with IFA. While the IFA titers drop again steadily after approximately 15 weeks, the titers achieved with the microcapsule mixture remain relatively constant over a period of at least 28 weeks.

Example 4 describes the potentiation of the antibody response to the branched multiple antigen peptide of the designation P30B2 (in accordance with Fig. 1), constructed from a universal T_H-cell epitope of the tetanus toxin (sequence 947 to 967) and a B-cell epitope of the repetitive region of the circumsporozoite protein of Plasmidium berghei: analogously to Example 1, P30B2 was inserted into poly (d,1-lactic acid co-glycolic acid) 50:50 (Resomer RG502, Boehringer Ingelheim) and processed into spherical microparticles (RG502). The microparticles loaded with antigen were suspended in a 5% sterile solution of egg lecithin (Ovothin 170, Lukas Meyer, D-Hamburg) by shaking. This suspension was subcutaneously injected into a group of 8 BALB/c mice in respective amounts of 0.5 ml. The amount of antigen injected into each mouse was 3x10 µg. The injection was repeated after 16 days (first booster) and 113 days (second booster). A second group of 8 BALB/c mice were immunized with the same amount of antigen in incomplete Freund's adjuvant (IFA) in accordance with the same vaccination plan as a control. The antibody titers were determined by means of ELISA.

Fig. 5 shows the progression over time of the immune potentiation after booster injections with RG502 in comparison to IFA associated with Example 4. The antibody titers obtained from RG502 and IFA rise comparatively. Accordingly, the process in accordance with the invention is also suitable for the immune potentiation achieved by boosters.

Example 5 describes the potentiation of the T_H-lymphocyte proliferation on the multiple antigen peptide of the designation P30B2 in accordance with Examples 1 to 4. P30B2 was inserted into RG502, RG752 and R206 analogously to the Examples 1, 2 and 3 and processed into spherical microparticles of different degrees of expandability. Identical amounts of microparticles containing P30B2 were suspended in a 5% sterile solution of egg lecithin (Ovothin 170, Lukas Meyer, D-Hamburg) by shaking. This suspension was subcutaneously injected into a group of 8 BALB/c mice in respective amounts of 0.5 ml. The amount of antigen injected into each mouse was 30 µg. A second group of 8 BALB/c mice were immunized with the same amount of antigen in incomplete Freund's adjuvant (IFA) as a control. The T-cell proliferation in the lymph nodes was determined in a known manner.

Fig. 6 shows the T-lymphocyte proliferation described in Example 5 14 days after the administration of various microcapsule formulations as well as of an IFA preparation. It can be seen from this that all microcapsule formulations, i.e. RG502 with rapid antigen release, RG752 with an antigen release of medium slowness and R206 with greatly slowed antigen release, as well as the mixture of all three microcapsule types, all potentiate the T-lymphocyte proliferation in an at least comparable, partially greater extent than an IFA preparation.

Example 6 describes the triggering of a cytotoxic T-lymphocyte reaction of a T_C cell epitope of the circumsporozoite protein of Plasmodium berghei (CTL 359A, sequence 252 to 260): 0.008 g of CTL 359A were dissolved in 1.0 g of water and this solution was subsequently dispersed by means of an ultrasonic generator in a solution of 4.0 g poly (d,1-lactic acid co-glycolic acid) (Resomer 502, Boehringer Ingelheim) in 60.0 dichloromethane. Spherical microparticles were produced by means of spray drying. The microparticles loaded with CTL 359A were mixed with microparticles loaded with P30B2 in accordance with Example 1 in a ratio of CTL 359A ; P30B2 of 1 : 10 to increase the immune response to CTL. The mixture of the microcapsules was subsequently suspended in a 5% sterile solution of egg lecithin (Ovothin 170, Lukas Meyer, D-Hamburg) by shaking. This suspension was subcutaneously injected into a group of 2 BALB/c mice in respective amounts of 0.5 ml. The amount of antigen injected into each mouse was 4 μg of CTL 359A and 40 μg of P30B2. The T_C-cell response was determined after 10 and 20 days by means of a cell lysis test.

Fig. 7 shows the T_C-lymphocyte response associated with Example 6, which was determined 10 and 20 days after immunization, or administration of the formulations. The percentile cell lysis activity is represented as a function of the effect/target cell ratio E/T. In a surprising manner, the simultaneous administration of microencapsulated T_C epitope and T_H epitope (P30B2 + 359A in RG502) induces a significant T_C-lymphocyte stimulation which can be observed 20 days after administration. The progress over time of the T_C response which, in contrast to

the T_H and antibody response requires a considerably longer time, appears to be of particular interest.

It is essential for the invention that biodegradable spherical microparticles are proposed which potentiate the immune response to synthetic antigens. By determining the physico-chemical properties of the biopolymers used it is possible to control the extent and progress over time of this immune potentiation. The process furthermore makes it possible to also stimulate the cytotoxic L-lymphocytes in addition to the antibody and T_H-lymphocyte potentiation. The extent of immune potentiation is at least comparable with the potentiation achieved by means of IFA preparations and its progress over time is clearly prolonged. A process is therefore available which can be employed in the immunization of humans and animals against diseases caused by viruses, bacteria, protozoa or tumor cells.

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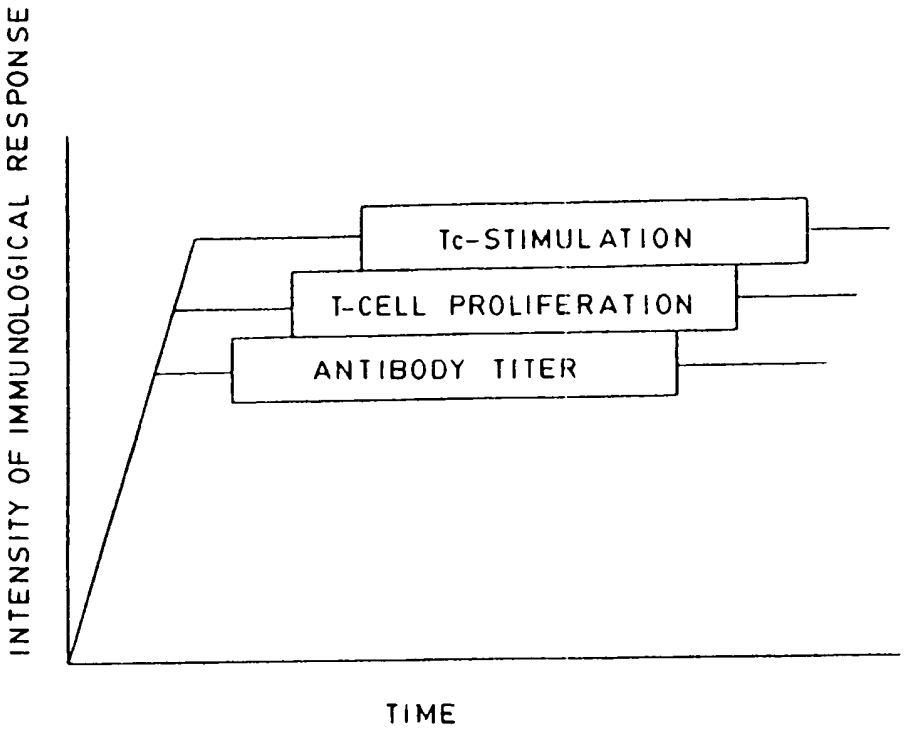

Fig. 1

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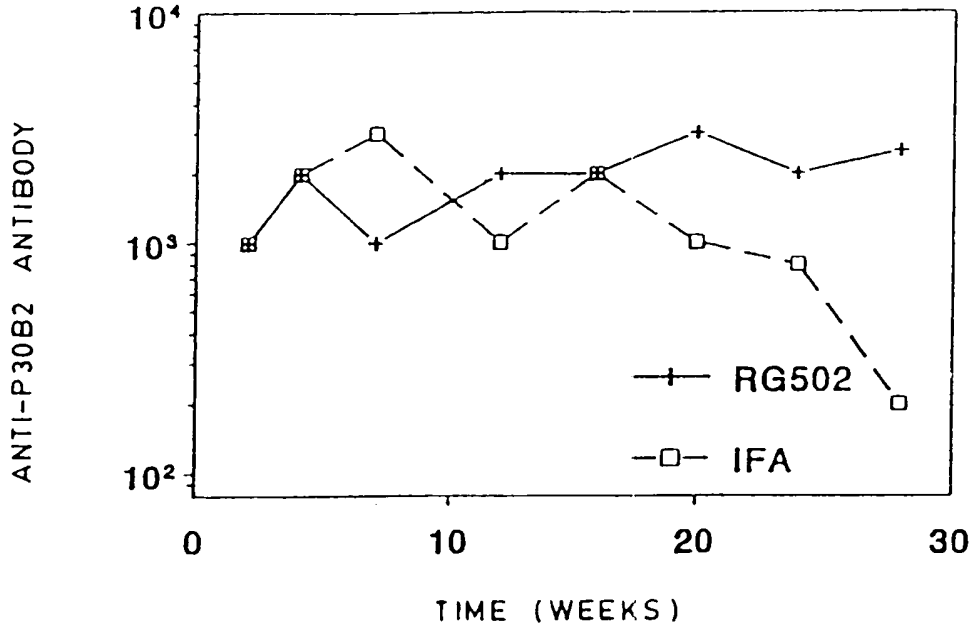

Fig. 2

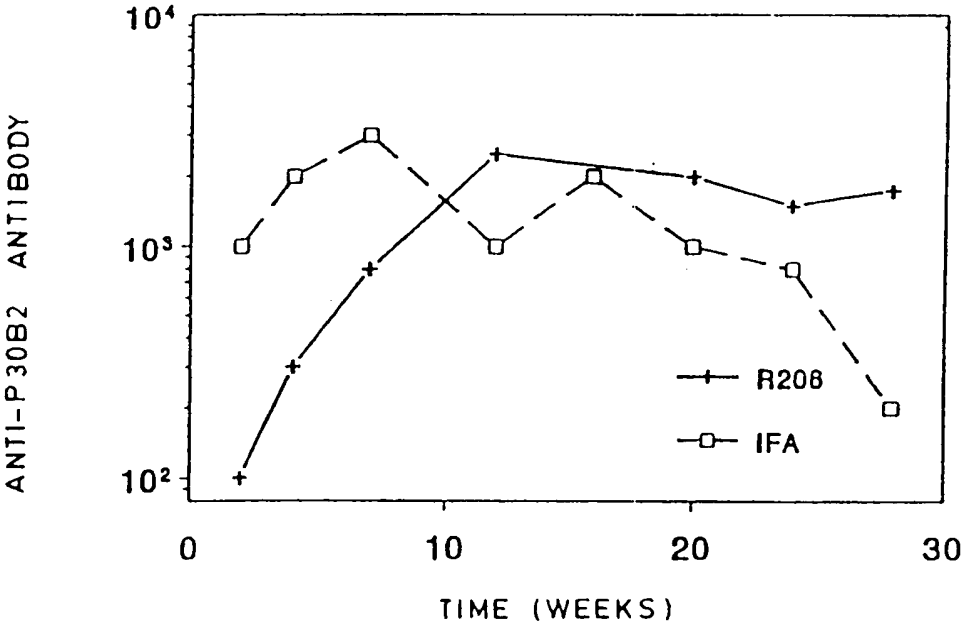

Fig. 3

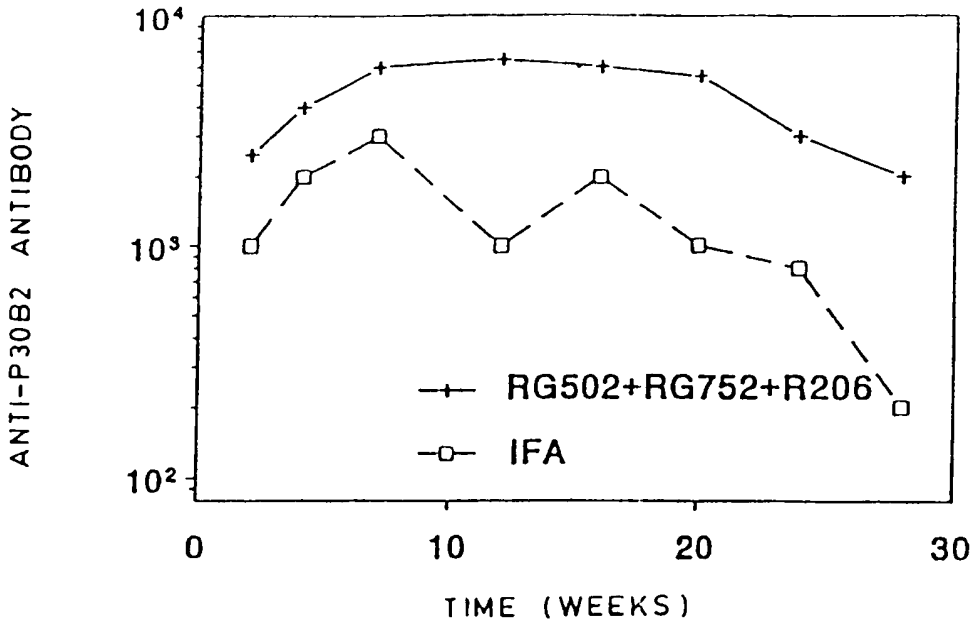
ANTI-P30B2 ANTIBODY
10^4
10^3
10^2
RG502+RG752+R206
IFA
0
10
20
30
TIME (WEEKS)

Fig. 4

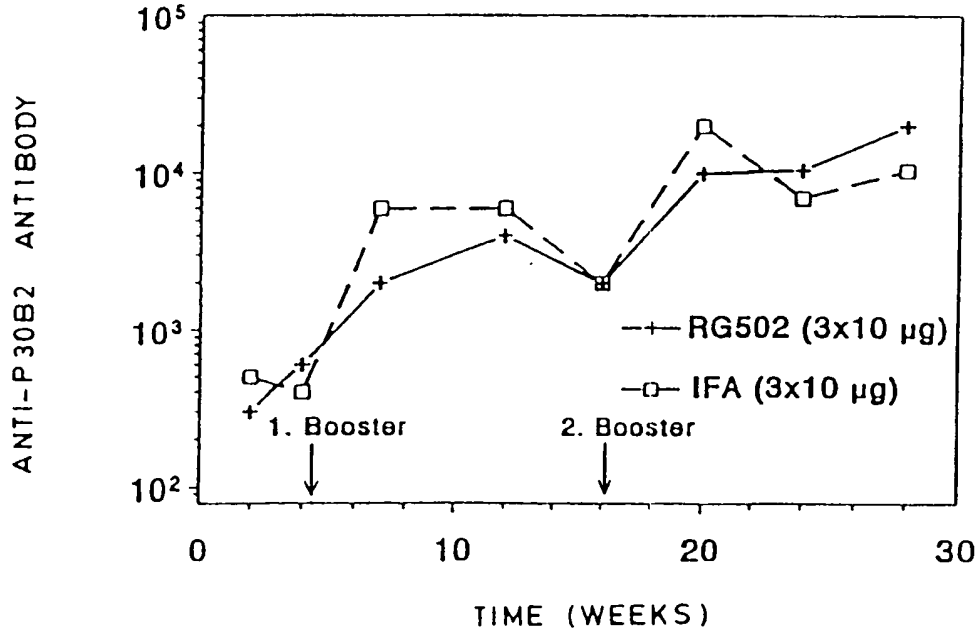
ANTI-P30B2 ANTIBODY
10^5
10^4
10^3
10^2
RG502 (3x10 µg)
IFA (3x10 µg)
1. Booster
2. Booster
0
10
20
30
TIME (WEEKS)

Fig. 5

2156718

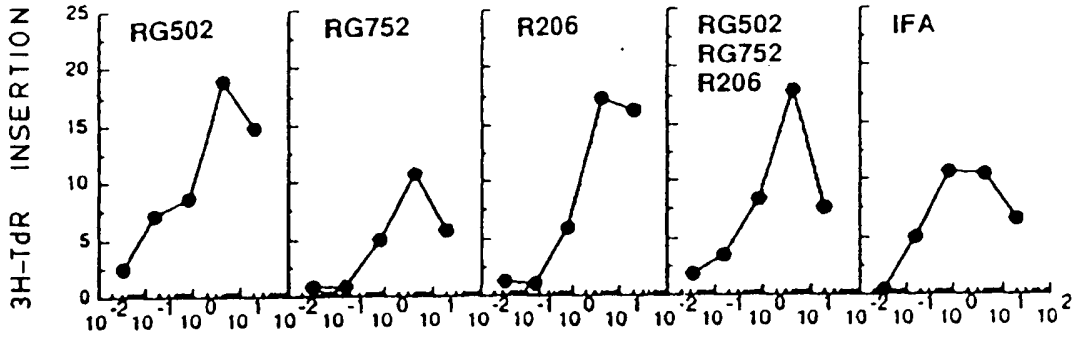

Fig. 6

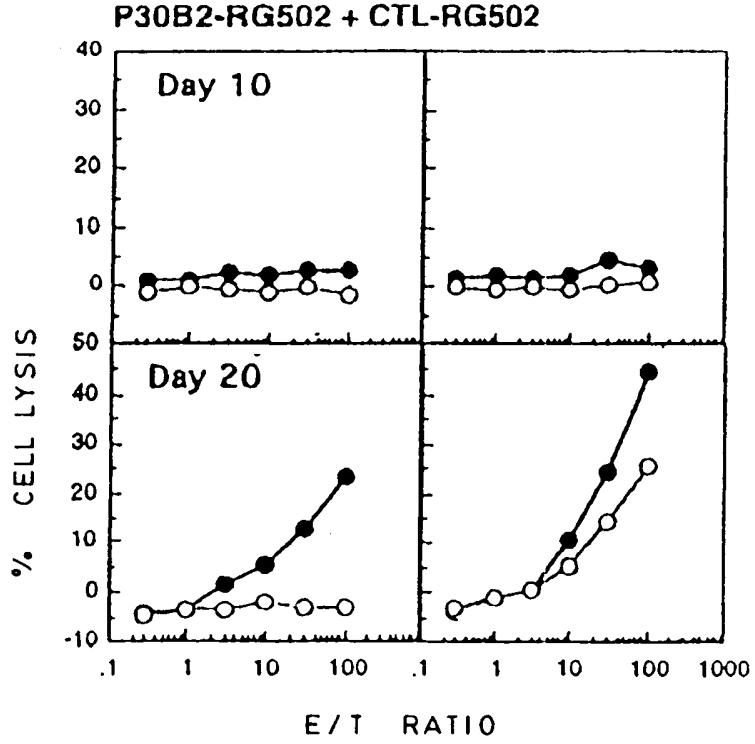

Fig. 7

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Ottawa Hull K1A 0C9

(21) (A1) 2,156,718

(86) 1994/12/23

(43) 1995/06/29

(51) Int.Cl.[6] A61K 39/39; A61K 39/00; A51K 9/16

(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Immunological Response Potentiation Process

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(30) (CH) 3849/93-6 1993/12/23

(57) 12 Claims